Tanzania Journal of Science 50(3): 433-446, 2024
ISSN 0856-1761, e-ISSN 2507-7961

Evaluation of therapeutic efficacy of *Aloe rabaiensis* leaf against *Salmonella gallinarum* challenged in Kuroiler chicks in Arusha, Tanzania

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Received 5 Jun 2024, Revised Aug 25 2024, Accepted Sept 7, Published Sept 30 2024
https://dx.doi.org/10.4314/tjs.v50i3.2

Abstract

Salmonella gallinarum, a bacterium causing fowl typhoid in gallinaceous birds, poses significant economic challenges to the chicken industry in Tanzania. This study investigated the potential of *Aloe rabaiensis* in managing *S. gallinarum* in Kuroiler chicks. The clinical signs, viable cell count, live weight, heterophils, lymphocytes, and monocyte counts were evaluated using ninety-four chicks grouped into T1, T2, T3 (challenged with *S. gallinarum*), and T4 (unchallenged with *S. gallinarum*). The findings revealed no statistically significant differences between the treated and control groups ($P > 0.05$) in viable cell count, live weight, mortality rate, heterophils, lymphocytes, and monocyte counts. However, there were clinical signs of fowl typhoid in the challenged groups (T1, T2, T3). The groups challenged with *S. gallinarum* and treated with *A. rabaiensis* (T1, T2) showed reduced susceptibility to the bacteria compared to the control group, T3. The mortality rate for T1 was 14.3% (4, n=28) and T2 17.9% (5, n=28), while the control group T3 was 25% (7, n=28). The lower mortality rates and less severe fowl typhoid symptoms in the *Aloe*-treated groups were attributed to the antibacterial efficacy of *A. rabaiensis* leaf against *S. gallinarum*, offering a promising remedy for the future of poultry health.

Keywords: *Salmonella gallinarum*; Kuroiler chicks; *Aloe rabaiensis;* Biomarkers

Introduction

Fowl typhoid is a bacterial disease of chickens, turkeys, ducks, and other gallinaceous birds of all ages (Ojima et al. 2022). The causative agent, *Salmonella gallinarum*, is among the reasons for mortality and morbidity in chicken production. The immense economic loss due to fowl typhoid is associated with diminishing egg production, which results in poor productivity and the deaths of about 100 % of highly susceptible poultry flocks (Markos and Abdela 2016). In Tanzania, fowl typhoid is second to Newcastle disease in causing mortality in local breeds of chickens (Nonga et al. 2010, Mngumi and Bunuma 2022). Drugs used to treat fowl typhoid include fluoroquinolones, sulfonamides, oxytetracycline, nitrofurazone, and ampicillin (Waihenya et al. 2002). Despite the drugs used to treat fowl typhoid exhibiting a reduction of clinical signs and mortality in chickens, there is still antibiotic resistance to *Salmonella* isolated from chickens (Kang et al. 2010, Amir et al. 2019). In addition, live attenuated vaccines by viruses or bacteria are genetically manipulated to reduce and weaken the pathogen's virulence (disease-inducing ability) against a host. However, the immunocompromised condition of a host, underdose of a vaccine and secondary mutations to either virus or bacteria used to make vaccines can cause reversion and virulence (Kumar 2021). Similar to this observation, live vaccines such as Gallivac^{R} SE, Nobilis® SG 9R, and TAD Salmonella

vac® E confer immunity against disease in chickens (Penha Filho et al. 2010, Koerich et al. 2018, Redweik et al. 2020). However, in some cases, immunisation retains some organisms' virulence, which may persist for months and raise the possibility of vertical transmission through the eggs (Kwon and Cho 2011). The contaminated eggs, in turn, accelerate the spread of fowl typhoid through hatched chicks. The challenges associated with commercial drugs and vaccines for controlling fowl typhoid necessitate looking for alternatives from medicinal plants.

The genus *Aloe* comprises more than 550 species, and the majority are known for their excellent treatment against different ailments of humans and animals in ethnomedicine and ethnoveterinary (Waihenya et al. 2002, Bjorå et al. 2015). The *A. rabaiensis* is among 44 *Aloe* species found in Tanzania, with its availability around Lake Jipe in northern Tanzania (Amir et al. 2019). The therapeutics of *Aloe* are attributed to the presence of aloin and emodin, which are the active constituents of various antimicrobial agents of *Aloe* (Joseph and Raj 2010). *Aloe's* bioactive compounds are essential for treating stomach ailments, gastrointestinal problems, constipation, wounds due to cuts and burns, dysentery, diarrhoea and the treatment of skin diseases (Kumar and Yadav 2014). A survey on ethnoveterinary and ethnomedical practices of *A. rabaiensis* among the Maasai and Pare communities revealed the plant to be useful against bacterial diseases associated with intestinal infections. In addition to this observation, cold water extraction for *A. rabaiensis* is used to manage an enlarged spleen in Kenya (Mutwiwa et al. 2018).

Medicinal plants of the genus *Aloe* have been proven to treat and prevent several diseases of human importance. However, little is known about the therapeutic potential of *A. rabaiensis* against bacteria associated with diarrhoea diseases in poultry. Therefore, the study evaluates the efficacy of *A. rabaiensis* against *S. gallinarum* challenged in Kuroiler chicks by observing clinical signs, viable cell count, live weight, heterophils, lymphocytes and monocyte counts.

Materials and Methods

Experimental chicks and their management

A study used ninety-four female Kuroiler chicks that were four weeks old and purchased from a local hatchery in Arusha, Tanzania. Before the experiment, the chicks were acclimatised for three weeks at the Livestock Training Agency (LITA) Tengeru, Arusha chicken house. During the three weeks, the chicks received multivitamins, antibiotics, and the Newcastle vaccine (LaSota® vaccine, BIOVAC Ltd, Israel). Also, the chicks received standard chick mash from Kibo feeds and water ad libitum.

Ethics permit

The study received approval from Kibong'oto Infectious Diseases Hospital (KIDH), Nelson Mandela African Institution of Science and Technology (NM AIST), and the Centre for Educational Development in Health (CEDH) research committee, with approval number KNCHREC006.

Preparation of *Aloe rabaiensis* powder

Aloe rabaiensis was obtained from around Lake Jipe in northern Kilimanjaro, Tanzania (3.34882 S, 37.44202 E at 718 M) from November to December 2020. The Botanist authenticated the plant from Tanzania Plant Health and Pesticide Authority (TPHPA), and voucher specimen ARH 403 was deposited in the herbarium at TPHPA. The collected leaves of *A. rabaiensis* were then washed with running tap water and rinsed with distilled water. Afterwards, the leaves were chopped into fine particles using a sterile sharp knife and dried in the oven (38 °C). After drying, the leaves were ground into fine particles by a mill machine (Swinging Traditional Chinese Machine Pulveriser Diaxiang electronic equipment, DXF- 20D, China) and stored in khaki food bags until use.

The amount of the powdered *A. rabaiensis* leaf mixed with the chick's feed was determined by taking six fresh leaves to weigh 1 kg, grinding them into fine particles and soaking them in 2 Liters of distilled water for 24 hours (28° C). The soaked plant materials were sieved using cotton wool. The filtrates were freeze-dried, and the extract

was collected and stored in tight bottles at 4 °C. The fresh leaves weighing a similar amount of 1 kg were then chopped into fine particles and dried under the oven (38° C) until dry. The two (freeze-dried extract and oven-dried leaf) were used to estimate the concentration of powdered *A. rabaiensis* leaf mixed with chick feed offered to chicks challenged with *S. gallinarum*.

Preparation of *S. gallinarum* (inoculum)

This study used the original stock of *S. gallinarum* isolated from a local breed of chicken during an outbreak of salmonellosis. Biochemical reactions identified the isolated colon, then sub-cultured in selective media on MacConkey agar at 37 ℃ for 24 hrs (Mkangara and Mpenda 2022). The pure colon from MacConkey was then enriched by subculturing it on nutrient agar. One colony from nutrient agar was selected and inoculated in nutrient broth (5 mL) in a universal bottle and incubated at 37 ℃ for 8 hrs in a shaker. The serial dilution was taken, and the pour plate method was used to determine the viable bacteria cell counts of the broth culture. In the meantime, the culture was kept in a refrigerator for the viable cell count to be known. During this time, the inoculated broth was diluted with phosphate-buffered saline (PBS) for an infective dose of 5.0×10^8 c.f.u/mL (0.5 MacFarland turbidity standard).

Experimental design

The investigation started by screening the chicks for antibodies against *S. gallinarum* using the Rapid Plate Agglutination Test (RPAT). The *S. gallinarum* antigen (Veterinary Laboratory Agent, New Haw, UK) was used for RPAT. The *Salmonella*-free chicks were randomly divided into four treatment groups: T1, T2, T3 and T4. The T1 was infected treated (28 chicks, 500 mg/mL ≡ feed with 83.3 g/kg of *A. rabaiensis*)), T2 infected treated (28 chicks, 300 mg/mL ≡ feed with 50 g/kg of *A. rabaiensis*), T3 infected untreated (28 chicks, standard feed with 0 g/kg of *A. rabaiensis*) and T4 uninfected untreated (10 chicks, standard feed with 0 g/kg of *A. rabaiensis*). The T1, T2 and T3 received by oral gavage into the crop 1mL of nutrient broth containing 5.0×10^8 c.f.u of *S. gallinarum* made by pour plate method. The negative control chicks were given 1 mL of sterile nutrient broth. Chicks were observed daily for clinical signs and mortality. After the first chick showed the clinical signs of fowl typhoid, 83.3 g and 50 g of pulverised *A. rabaiensis* (equivalent to 500 mg/mL and 300 mg/mL of freeze-drying *A. rabaiensis*, respectively) were mixed with standard chick mash (1 kg each). The standardised chick mash added 83.3 g/kg and 50 g/kg of *A. rabaiensis*, were then, offered to challenged treatments with *S. gallinarum* T1 and T2, respectively, for fourteen consecutive days.

Clinical sign and mortality observations

The clinical signs and mortality in chicks during the experiment were monitored daily, and each chick was examined separately. The scoring system used to record clinical signs was that used by Christensen et al. (2003) and Christensen et al. (1996) with + referring drowsy, ++ referring drowsiness, ruffle feathers, occasional closure of the eyes and +++ for closure of the eyes, reluctance to move, creamy whitish diarrhoea from the vent. The number of chicks affected was recorded using the scores, and each group's mortality was recorded.

Bodyweight, pathological changes and viable cell count

The body weight records of chicks from all treatments were taken using the standard weighing balance on days 0, 3, 6, 9, 12, and 15 post-infections. Dead and sacrificed chickens undergo post-mortem examination for pathological changes related to fowl typhoid. Two chicks with no clinical signs selected randomly from each group were sacrificed on day 3 post-infection. Three chicks from each group with severe clinical symptoms were then sacrificed on days 6 and 9, and the remained chicks from each group were sacrificed on day 15 post-infection. The viable cell count was performed as follows: -

The liver and spleen were hygienically excised from sacrificed chicks and weighed 1g each, then placed separately in sterile stomacher® bags and homogenised with 1mL of phosphate-buffered saline (PBS) for 3 min. Subsequently, 0.5 millilitres of the

homogenate was serially diluted 10-fold in PBS (added to make a total of 10 mL), and 0.5 mL of each dilution was spread on a MacConkey agar plate and incubated at 37 °C for 24 h. After the incubation period, the number of colonies grown in each plate was recorded. The record was done for plates with colonies ranging between 30 and 300.

Determination of differential white blood cell count- leukocytes

Three chicks were randomly picked from each treatment on days 0, 3, 6, 9, 12 and 15 to collect blood to make a thin microscopic blood film for leukocyte count. The thin blood film on the microscopic slide was air-dried and stained using Wright's staining method (Drijver and Boon 1986). The stained films were observed under a light microscope (1000× magnification), and the white blood cells (heterophils, monocytes, lymphocytes, basophils and eosinophils) were counted according to their morphological characteristics. The white blood cell differential counter was then used to record 100 cells counted from each slide. However, this study did not consider the number of basophils and eosinophils because they are biomarkers for parasitic infections and allergies, not bacterial infections. The results were expressed as percent distribution (p.d).

Data analysis

Mean viable bacterial cell count datasets were analysed using the Minitab Statistica Software 13.20 © 2000 Mintab Inc PA 16801-9928, USA. Means were separated using Tukey's test. Values were considered statistically significant at $P < 0.05$. The Microsoft Excel programme performed the graphical analysis of the mean per cent distributions of heterophils, lymphocytes and monocytes.

Results

Clinical symptoms

Symptoms associated with fowl typhoid were revealed on day 3 post-infection (PI) for infected treated group T2 and infected untreated group T3 with two chicks (9.5%) and four chicks (19%), respectively, found drowsy. No chick showed clinical symptoms on day 3PI to infected treated group T1 contrary to T2 and T3. There was no creamy whitish diarrhoea in either treated group, T1 and T2, which received feed with 83.3 g/kg and 50 g/kg of *A. rabaiensis*, respectively. The results for daily scoring of clinical symptoms are summarised in Table 1.

Table 1: Scoring of the clinical symptoms of fowl typhoid caused by *S. gallinarum* according to Christensen et al. (1996)

DPI	T1	T2	T3	T4
3	-	(2) +	(4) +	-
4	(3) +	(2) +	(4) +, (1) ++	-
5	(3) +, (1) ++	(1) +, (3) ++	(4) ++, (1) +++	-
6	(2) +, (2) ++	(4) +, (2) ++	(3) ++, (3) +++	-
7	(5) ++	(3) ++	(4) +, (1) +++	-
8	(2) ++	(2) ++	(4) +, (2) +++	-
9	(3) +, (2) ++	(2) +, (2) ++	(4) +, (3) +++	-
10	(2) ++	(2) +, (2) ++	(1) -, (4) ++, (2) +++	-
11	(2) ++	(2) ++	(1) -, (4) ++, (2) +++	-
12	(4) ++	(3) +, (1) ++	(1) -, (4) ++, (3) +++	-
13	(2) +, (3) ++	(2) ++	(1) -, (4) ++, (2) +++	-
14	(2) + (1) ++	(2) +, (3) ++	(1) -, (3) +++	-
15	(2) +, (2) ++	(2) ++	(1) -, (2) +++	-

T1-T4 are experimental groups; T1 infected treated (83.3 g/kg); T2 infected treated (50 g/kg); T3 infected untreated (0 g/kg); T4 uninfected untreated (0 g/kg). +, drowsy; ++, drowsiness, ruffle feather, occasional closure of the eyes; +++, closure of the eyes, reluctant to move, white-yellowish diarrhoea from the vent; -, no clinical symptoms; ( ) number of chickens with specific signs; DPI refers days post-infection.

Leukocytes variation

Heterophil count

The infected untreated group T3 showed the highest mean percent distribution (pd) for heterophil count compared to other groups to day 3. Then, decreased steadily to day 15 post-inoculation, as seen in Figure 1. The two infected treated groups, T1 and T2, had a variation in heterophil count to day 3 and decreased to day 6, and the decrease was almost similar from day 9 to day 15 post-challenge. The uninfected untreated group T4 showed the lowest heterophil count throughout the experiment. However, on day 15 post-challenge, the group coincided with the two treated groups, T1 and T2. In comparing the means, there were no statistically significant differences between the treatment groups ($P > 0.05$).

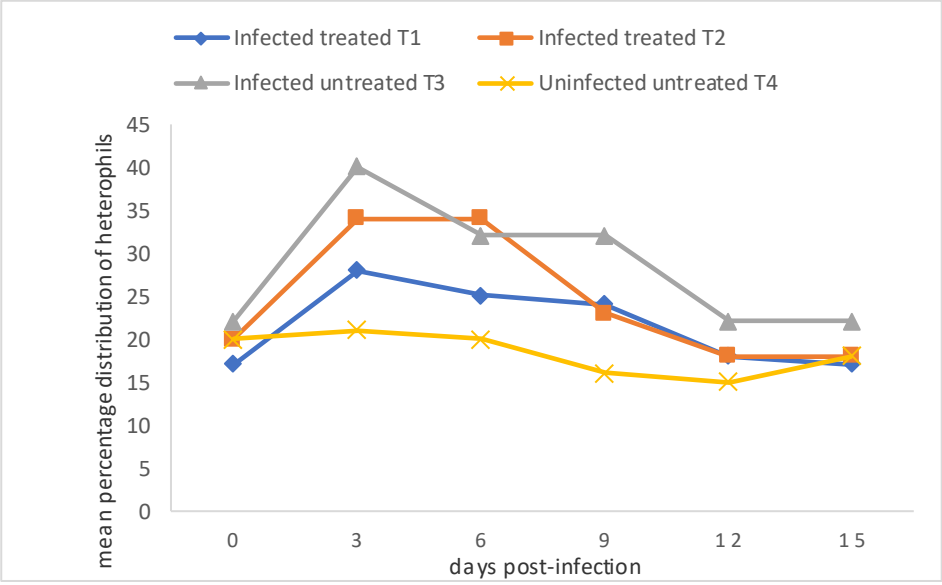

Figure 1: Change in mean percent distribution (pd) of heterophils (white blood cells component) as a biomarker against antigens in chicks challenged with *S. gallinarum* and treated with *A. rabaiensis* leaf

Lymphocytes

Figure 2 shows the mean per cent distribution (pd) for lymphocytes. The pd for lymphocytes to infected groups T1, T2 and T3 varied slightly from one another. No statistically significant differences ($P > 0.05$) were observed after comparing the means for all groups T1, T2, T3, and T4. The uninfected untreated group T4 had the lowest pd for lymphocytes compared to other groups. The infected treated groups T1 and T2 decreased in pd for lymphocytes from day 9 to day 15.

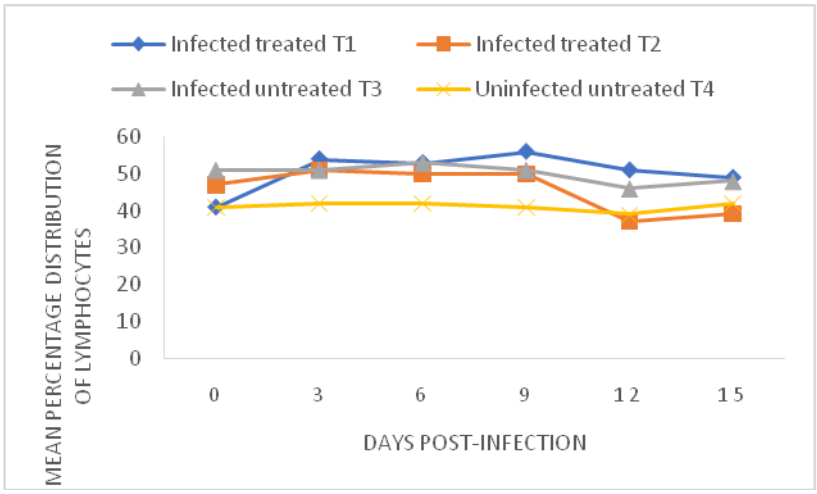

Figure 2: Change in the mean percent distribution (pd) of lymphocytes (white blood cell component) as a biomarker for stress in chicks challenged with *S. gallinarum* and treated with *A. rabaiensis* leaf

Monocytes

The mean pd for monocytes is shown in Figure 3. There was a sharp decrease of mean pds for monocytes on day 3PI to infected treated T1, T2, and infected untreated T3. The uninfected untreated T4 slightly decreased monocyte count, while T3 demonstrated the lowest pd for monocytes throughout the experiment. The findings also observed increased mean pd for monocytes from day 9 to 12 for all treatments T1, T2, and T3. However, there was a slight decrease in mean pd for monocytes on days 12 to 15 for treatment groups T2 and T3. In comparing the means, there was no statistical significance difference ($P > 0.05$) between the groups.

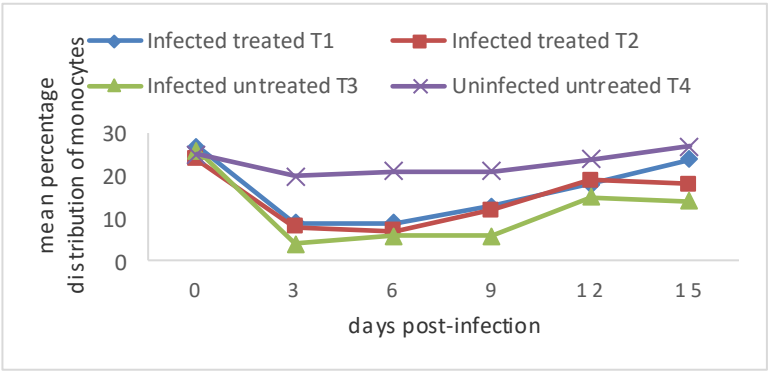

Figure 3: Change in mean percent distribution (pd) of monocytes (white blood cell component) as a biomarker for influencing the adaptive immune response in chicks challenged with *S. gallinarum* and treated with *A. rabaiensis* leaf

Mean viable bacteria cell count for liver and spleen

The change in the mean viable bacteria cell counts (log10) in the liver and spleen for treatments T1, T2, and T3 is not statistically significant ($P > 0.05$). The viable cell counts in the liver and spleen of T1, T2, and T3 increased to the maximum on day 9; however, on day 15, there was a residual

bacterial count with significance ($P < 0.05$) for treatment T1 and T2, as seen in Table 2.

Table 2: Mean viable bacterial cell count of *S. gallinarum* in both liver and spleen tissue expressed in $\log_{10}$ after treatment with *A. rabaiensis* leaf

Organ	Days	T1 (Infected treated)	T2 (Infected treated)	T3 (Infected untreated)	F - statistic	P - value
Liver	3	2.775 ± 0.395	2.885 ± 0.545	3.525 ± 0.325	0.881	0.5
	6	4.507 ± 0.237	4.590 ± 0.340	5.346 ± 0.052	3.676	0.091
	9	5.340 ± 0.254	5.437 ± 0.213	6.160 ± 0.124	4.805	0.057
	15	0.983 ± 0.983	1.283 ± 0.641	3.087 ± 0.052	2.811	0.138
Spleen	3	2.670 ± 0.410	2.86 ± 0.460	3.155 ± 0.045	0.469	0.664
	6	4.587 ± 0.357	4.737 ± 0.293	4.783 ± 0.191	0.127	0.883
	9	5.413 ± 0.249	5.373 ± 0.264	6.090 ± 0.070	3.565	0.095
	15	0.680 ± 0.068	1.233 ± 0.623	3.153 ± 0.026	5.941	0.037

Infected treated T1 (83.3 g/kg), Infected treated T2 (50 g/kg), Infected untreated T3 (0 g/kg)

Body weight and pathological lesions

Figure 4 shows the body weight changes of chicks over the experimental period. The uninfected untreated T4 gradually increased weight from day 3 to day 15. Unlike the treated group, T1, there was a slight decrease in weight from day 6 to 9 and an increase on day 12 to 15. In T2, there is an equilibrium between day 6 and day 9, with a slight decrease in day 15. In infected untreated T3, there is a steady decrease in weight from day 3 to day 15. There is no statistical significance in the body weight of the treatment groups ($P > 0.05$).

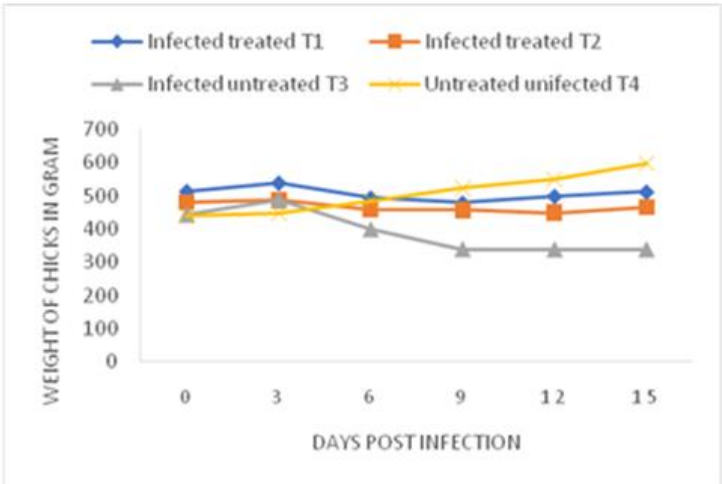

Figure 4: Trend in the change of body weights to the treatment groups T1, T2, T3, and T4 received *A. rabaiensis* leaf formulated feed.

The post-mortem examination of the dead chicks and sacrificed chicks evidenced pathological changes with the characteristics of fowl typhoid. The factors include an

enlarged liver and spleen; the liver showed patches of cooked appearance with necrotic foci, Figure 5. The necrotic foci were also observed in the spleen and myocardium. Other features seen in dead chicks include slimy intestinal contents in the small intestine, dark brown bone marrow, and anaemic and fibrinous pericarditis. However, there were slightly clear-cut differences in the severity of lesions between the *Aloe*-treated (T1 and T2) and negative control groups T3.

Figure 5: Enlarged liver of a post-mortem chick with characteristics of fowl typhoid

Mortality rates

The trend of cumulative mortalities for experimental groups T1, T2 and T3 are shown in Figure 6. Mortality was first observed on day 6 in infected untreated group T3 with the death of four chicks (4/28 (14.3 %)), and infected treated groups T2 and T1 with the death of two chicks in each treatment (2/28 (7.1 %)). By day 15, the cumulative mortalities of infected untreated T3 was 7/28 (25%), infected treated T2 5/28 (17.9%) and infected treated T1 4/28 (14.3%). In T1, there was no increase in mortality from day 12 to day 15, while in T2 and T3, there was an increase in mortality. There was no statistical significance in cumulative mortalities ($P > 0.05$).

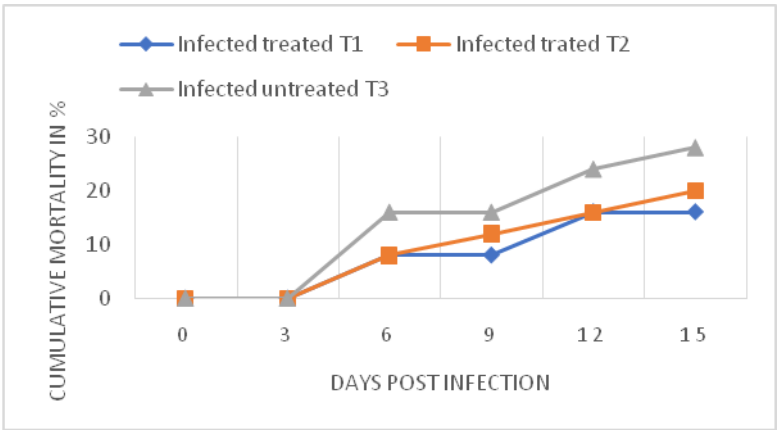

Figure 6: Trends of change in cumulative mortality rates of treatment groups T1, T2 and T3 of chicks infected with *S. gallinarum*

Discussion

The *A.* rabaiensis was used to manage *S. gallinarum* challenged to the Kuroiler chicks. The *A. rabaiensis* is among the *Aloe* spp., with several bioactive compounds used for prophylactic and therapeutic potential against human and animal ailments and in cosmetics worldwide (Khan et al. 2022). Regarding treatments with *Aloe* spp., *Aloe vera*, at a rate of 1.5%, was proven to be an effective dietary supplement than antibiotic growth promoter enramycin at enhancing broiler performance and lowering intestinal *Escherichia coli* and *Salmonella* spp. (Islam et al. 2020). The creamy whitish diarrhoea was observed in infected untreated group T3, which did not receive *Aloe*-treated feed on day 5 post-infection. The *Aloe*-treated feed chicks T1 and T2 did not show the clinical signs of whitish diarrhoea for the whole treatment period. The study of Singh et al. (2017) observed that the *Aloe*-treated feed reduces detrimental bacteria in broilers and promotes beneficial bacteria *Lactobacillus*, well known for enhancing nutrient digestion and absorption in the gut. A study by Budai et al. (2013) detected a higher immunomodulatory response in patients who received *Aloe* gel after it improved gastrointestinal motility, regulated intestinal pH and decreased stool transit, leading to the curing of the disease and elimination of clinical signs.

The post-mortem examination of *Aloe*-treated groups revealed that the entire intestinal content contained *Aloe* materials. Based on this fact, *Aloe* is a laxative agent that induces tight junction opening (Salehi et al. 2018). In doing so, the active ingredients of *Aloe* enhance the reduction of intestinal water absorption after increasing water retention in the intestinal tube. Water retention improves the solubility and bioavailability of phytoconstituents in the intestinal mucosa (Singh et al. 2013). The phytoconstituents strengthen the host-pathogen resistance by favouring intestinal microbiota, which later clears the pathogens through regular bowel movement (Willing 2019). Conversely, the study observed an increase in the survival rate of chicks from *Aloe*-treated groups T1 and T2. The efficacy of *Aloe* could be associated with the potencies mentioned above in inhibiting diarrhoea and other critical clinical signs of fowl typhoid. The maximum cumulative mortality of the chicks in infected untreated group T3 was 25%, while in *Aloe*-treated groups T1 and T2, were 14.3% and 17.9%, respectively.

The viable bacterial cell counts in the liver and spleen were used to check for bacterial load in the organs after the chicks had received treatment with *A. rabaiensis*. The increase in bacterial load Table 2, and the decrease in the weight of chicks reflected the health status as concluded by pathological observations of sacrificed chicks. Enlargement, bronze discolouration, and greyish necrotic foci or necrotic patches were observed on the livers of dead chicks, contrary to the control group T4. Splenomegaly and multiple necrotic foci on the surface were observed on the spleen. These findings coincide with Kumar et al. (2013) who observed bronze discolouration of the liver, splenomegaly and necrotic foci on the liver, spleen and heart of broilers infected with *S. gallinarum.* This study chose the liver and spleen because these are among the organs where *Salmonella* resides during systemic infection and keeps circulating in the blood systems. Understanding the changes in white blood cells (heterophils and monocytes) upon infection, the study observed a significant decrease in the percent distribution (pd) of these cells to challenged chicks treated with *A. rabaiensis.*

The viable bacteria cell count increased steadily from day 3, reached the maximum on day 9, and decreased with residual count on day 15. The residual count of bacterial cells on day 15 PI is probably associated with the bioactivity of *A. rabaiensis* in clearing *Salmonella*. The *Aloe*'s active ingredients, such as anthraquinone, polysaccharides, chromone, aloen and phytosterol, possess immunomodulatory properties that stimulate macrophages and other immune cells to enhance immune response. The

anthraquinones are comparable to prebiotics, which increase the *Lactobacillus* spp. colonies and decrease Gram-negative bacteria, including *Salmonella* (Darabighane et al. 2012). Other scholars observed peak viable bacteria count on day 10 with clearance on day 14 (Mdegela et al. 2002, Msofe et al. 2006). In contrast to these findings, Waihenya et al. (2002) observed peak bacterial count on both spleen and liver tissue on day 6 and day 7, respectively. However, in a similar study, the clearance of bacteria in the two organs from treated groups with *Aloe secundiflora* was observed on day 15 post-infection. The difference between Waihenya et al. (2002) and the current study on the peak of the viable cell count might be due to genetic differences between the chicks or the strain of the bacteria used.

The percentage distribution (pd) of heterophils increased to day 3PI in infected untreated group T3, infected treated groups T1 and T2, lowered on day 12, and remained constant to day 15 PI. This is because the heterophils are the first line of defence in avian, similar to neutrophils in mammals. Upon infection, heterophils peak up to act against the pathogens and are always higher in phagocytic reactions in infected chickens than in healthy ones (Harmon 1989, Farnell et al. 2003). The findings corroborate (Chiang et al. 2008), who observed the large influx of heterophils to the intestines of *Salmonella enteritidis*-infected chickens and contributes to defending against microbial infection. However, the immunological parameters cannot interpret the physiology and behavioural factors influencing host immune reactions. Based on this study, keeping the chicks in cages and inducing pain during blood collection for white blood cell count stressed the chicks and might have the consequences of lowering an immune response by heterophils.

Other studies have observed that the clearance of pathogens depends on phagocytosis and the microbicidal mechanism of the heterophils (Andreasen et al. 1991). Chicks' heterophils rely primarily on non-oxidative microbicidal mechanisms. The activated heterophils generate toxic reactive oxygen species (ROS) against bacteria such as *Salmonella* at the invasion site by releasing bactericidal proteins and proteolytic enzymes to perform phagocytosis (He et al. 2003). This is because when the heterophils are exposed to phagocytic stimuli, they undergo the respiratory bust and oxidised glucose but fail to produce increased hydrogen peroxide or superoxide anions (Guriec et al. 2018). Despite the absence of an efficient oxidative microbicidal mechanism, heterophils are quite effective in killing bacteria. The fact is that heterophils contain chicken host defence peptides known as avian beta-defensin 1 (AβD1) and avian-beta-defensin 2 (AβD2), formally known as gallinacin 1 and gallinacin 2 (Cuperus et al. 2013, Su et al. 2017). Gallinacins are components of the innate immune system that invoke an immediate immune response against harmful pathogens (Rengaraj et al. 2018).

The chicken infected with bacteria responds to pathogens by circulating monocytes in the blood to the affected sites, including organs and tissues. The circulating monocytes are then differentiated into functional macrophages and phagocytise the bacteria (Wei et al. 2018). Based on this fact, the pd of monocytes count on day 0 PI was higher and decreased gradually to day 3PI for T1, T2 and T3. The combined effects of *A. rabaiensis* treatment against *S. gallinarum* and acquired immunity conferred by monocytes, heterophils and lymphocytes significantly decreased the bacterial cell count in the liver and spleen, as observed in Table 2.

The biomarkers heterophils and lymphocytes play a significant role in innate and adaptive immunity (Thiam et al. 2022). The rise of heterophils during bacterial infection indicates how an active chicken manages the infection; however, the increase in lymphocytes is mostly revealed during viral infection, environmental stress and respiratory and cardiovascular diseases (Sun et al. 2008). Nevertheless, the significant increase in bacterial load from day 6 to day 9 indicates how the bacteria

escape the immune response of macrophages residing in the liver and spleen. However, the decrease of bacterial load observed on day 15 PI might be associated with the therapeutic potential of the *A. rabaiensis* against *S. gallinarum* challenged in experimental chicks or the vigour immune response of chicks against infection. Concurrent to this study, Msoffe et al. (2006) observed no bacterial load in the liver and spleen of Mbeya and N'zenzegere ecotypes challenged with *S. gallinarum* during the entire experiment period (14 days). The vigour of these ecotypes could have been caused by the adaptive immune response mediated by lymphocytes against the strain of bacteria used. Moreover, due to immunological memories, the adaptive immune system provides a more versatile means of defence, which increases protection against subsequent reinfection with the same pathogen (Janeway et al. 2001, Deets and Vance 2021). Nevertheless, antibodies migrating upon infections bind to antigens in bacteria, preventing adherence on mucosa surfaces and other important steps of infections or blocking receptor-ligand formation of toxins (Seixas et al. 2022).

Conclusion and Recommendations

The antibacterial efficacy of *A. rabaiensis* against chicks challenged with *S. gallinarum* was revealed after decreasing bacterial cell counts and clinical signs of fowl typhoid. The response of heterophils peaked on day 3 post-infection and decreased to day 15, indicating that the standard chick feed mixed with *A. rabaiensis* had significant efficacy against *S. gallinarum*. The chick feeds added *A. rabaiensis* contain secondary metabolites and fibre, essential for promoting digestibility and host-pathogen resistance. The fibre contents minimize the intestinal epithelial attachment sites for pathogens while promoting the attachment of beneficial bacteria. Failure of *Salmonella* to attach to the intestinal mucosa eventually prevents internalization into the *Salmonella*-containing vacuoles (SCV), a modified phagosome that initiates systemic infection. Failure to induce systemic infection, *Salmonella* remains in the intestinal tube, eliciting gastroenteritis and finally sheds out through feces. Additionally, *A. rabaiensis* mixed in chicks' feed promotes beneficial bacteria that improve intestinal health and performance and, lastly, enhance the good health status of the chicks, as observed from this study.

The study did not isolate and test pure compounds from *A. rabaiensis* leaf for antibacterial efficacy against *S. gallinarum*. Therefore, we recommend testing pure compounds in *A. rabaiensis* in vitro and in vivo against *S. gallinarum* and other chicken ailments for pharmacological investigations.

Acknowledgements

The author acknowledges Dr Zemu Mbilu of the Sokoine University of Agriculture (SUA) and Mr Denis Luwomba of Tanzania Veterinary Laboratory Agency (TVLA) for their professional and technical support.

Declaration of Competing Interest

The author declares no conflict of interest.

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